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DETERMINATION OF VITAMIN B_6 BY MEANS OF DIFFERENTIAL SPECTROPHOTOMETRY IN PHARMACEUTICAL PREPARATIONS IN THE PRESENCE OF MAGNESIUM COMPOUNDS

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Abstract: The content of pyridoxine hydrochloride in two-component pharmaceutical preparations containing various magnesium compounds was examined. The UV differentiation spectrophotometry was devised and compared with the reference method of high performance liquid chromatography (HPLC). The analysis of the absorbance spectra (A) and its first (D^1) and second (D^2) derivatives made it possible to establish the appropriate analytical wavelengths (A: 290 nm; D^1: 302 nm; D^2: 308 nm). It was proved that spectrum differentiation significantly corrects errors resulting from overlapping background especially when the magnesium hydroaspartate, lactate or magnesium lactogluconate is present together with vitamin B_6.

Keywords: derivative spectroscopy, vitamin B_6, pharmaceutical preparations

Every year, new vitamin preparations with different qualitative and quantitative compositions appear on the pharmaceutical market. Vitamin B_6, also called pyridoxine, occurs in three active forms: pyridoxol, pyridoxal and pyridoxamine, all of which undergo reversible transformation in the body. It takes part in the nitrogen metabolism of linoleic and linolenic acid, neurotransmitters (norepinephrine, dopamine, GABA, serotonin, histamine) and also carbohydrates. Pyridoxal phosphate (PALP) takes part in the synthesis of aminolevulinic acid, the precursor of heme porphyrin ring. Therefore, vitamin B_6 deficiency may result in anemia, as well as in neuropathy and depression (1-4).

Physicochemical properties of the active substances and the reactivity of groups, which the analyzed molecule consists of, are used in the quantitative analysis of pharmaceutical preparations. The alkaline properties of pyridoxine have been used in its acidimetric determination in an anhydrous environment consisting of a mixture of acetic acid and acetic acid anhydride (5–7). Other sources claim that it is possible to determine pyridoxine hydrochloride using the VIS spectrophotometry method after a color reaction with 2,6-dichlorochinochlorimide (8). The presence of a chromophore group also creates a basis for using the UV spectrophotometric analysis in the determination of this substance in pharmaceutical preparations of both tablets and injections (5, 7, 9). Analyses of B_6 vitamin by means of the capillary electrophoresis (10, 11) method and high performance liquid chromatography using a UV detector have also been described in the literature (7, 9, 12–17).

In the analysis of mixtures where bands of individual substances are weakly separated, the method of spectral differentiation using a spectrophotometer equipped with a special electronic system, which makes it possible to obtain such a spectrum by an ordinary absorbance measurement, is used more and more frequently (18). Secondary spectra are used to rapidly determine the compound identity, e.g., isoenzymes of pyruvate kinase, and in determining the differences in protein composition (18). They have also been successfully used to determine drugs in pharmaceutical preparations, plasma and serum. In pharmaceutical preparations, this method has been used to determine, e.g., the mixture of furosemide and spironolactone in capsules (19), indapamide and captopril (20), cinnarizine and nicergoline (21), naproxen and salicylic acid in serum (22) and theophylline in the presence of phenobarbital in plasma (23). Among numerous applications of this method, the possibility of simultane-

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ous determination of α- and β-acids in hop cones can be mentioned, where it can be successfully used without the complicated method of isolation from an extract (24). The differential spectrophotometry exhibits sensitivity to the presence of weak and overlapping bands and makes it possible to eliminate the background. Therefore, it becomes more and more useful in pharmaceutical analysis.

The aim of this study was to devise a UV spectrophotometric method using differential spectra of pyridoxine hydrochloride in pharmaceutical preparations also containing qualitatively and quantitatively different magnesium compounds as active substances. The results were evaluated by comparing them with the results obtained in the simultaneous determination using the reference method of HPLC.

EXPERIMENTAL

Materials and methods

Pyridoxine hydrochloride (vitamin B_6) manufactured by Sigma-Aldrich Chemie GmbH (Germany) was used as a reference substance. Selected pharmaceutical preparations available at pharmacies containing active substances, that were a mixture of pyridoxine hydrochloride and magnesium in various chemical forms (Table 1), were marked with the following symbols FB, LB, MB, PB and SB for the purpose of this study. These preparations differed in the composition and pharmaceutical form: tablets, coated tablets, lozenges, tablets for enteral administration (enteric-coated). All chemical reagents were p.a. grade.

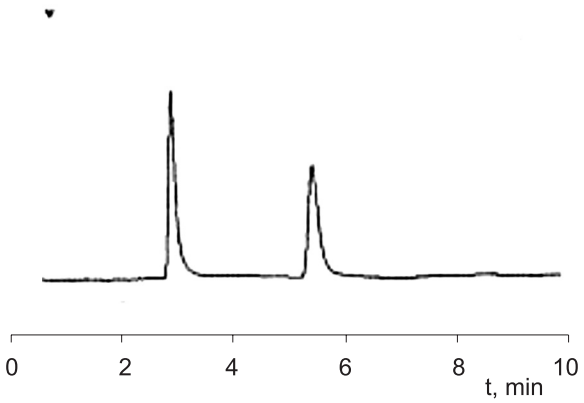

Figure 1. Typical HPLC chromatogram of 100 mL injection of mixtures of pyridoxine hydrochloride (t_r = 2.8 min) and internal standard (t_r = 5.3 min)

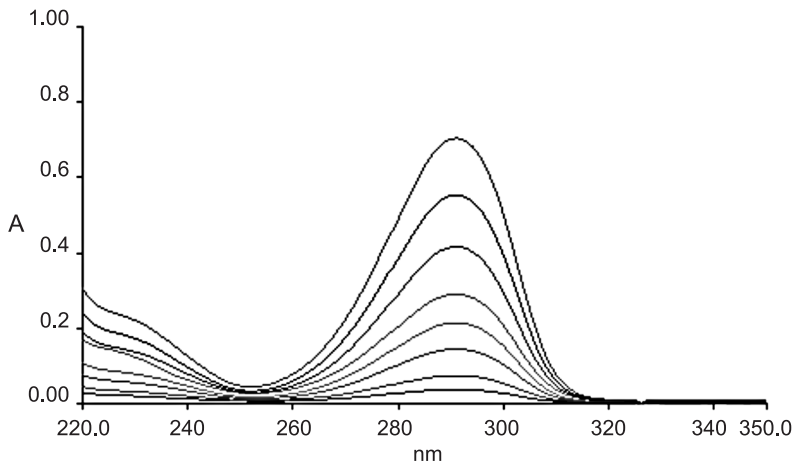

Figure 2. Zero derivative of the ratio spectra for different concentrations (1.0 – 20.0 μg/mL) of pyridoxine hydrochloride in the hydrochloric acid (0.1 M)

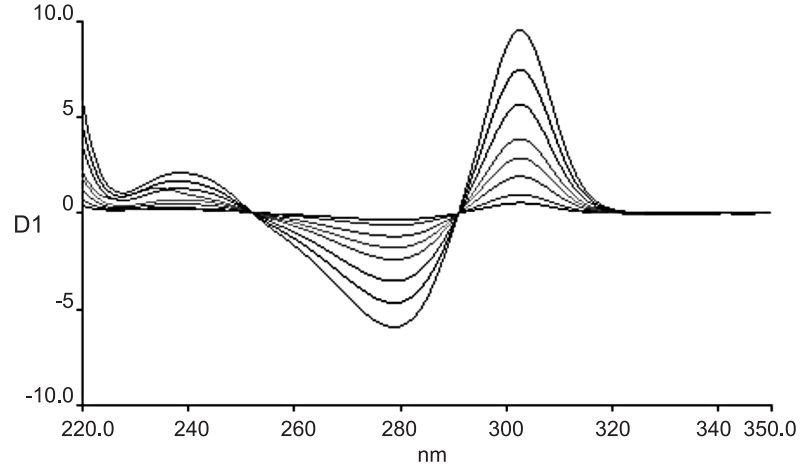

Figure 3. First derivative of the ratio spectra for different concentrations (1.0 – 20.0 μg/mL) of pyridoxine hydrochloride in the hydrochloric acid (0.1 M)

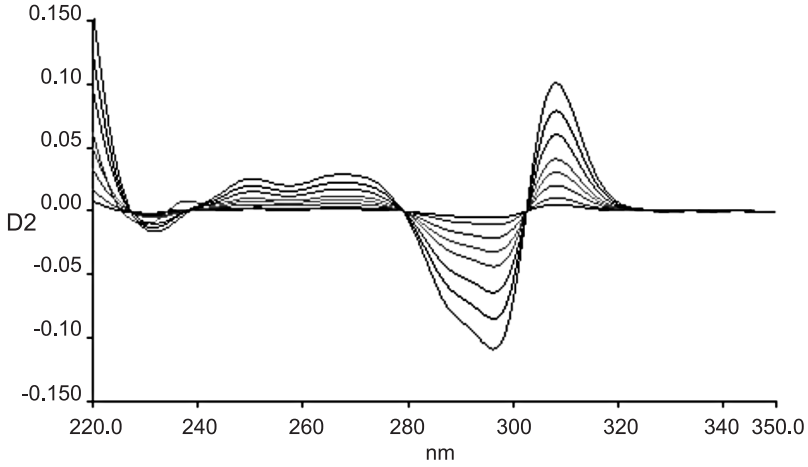

Figure 4. Second derivative of the ratio spectra for different concentrations (1.0 – 20.0 μg/mL) of pyridoxine hydrochloride in the hydrochloric acid (0.1 M)

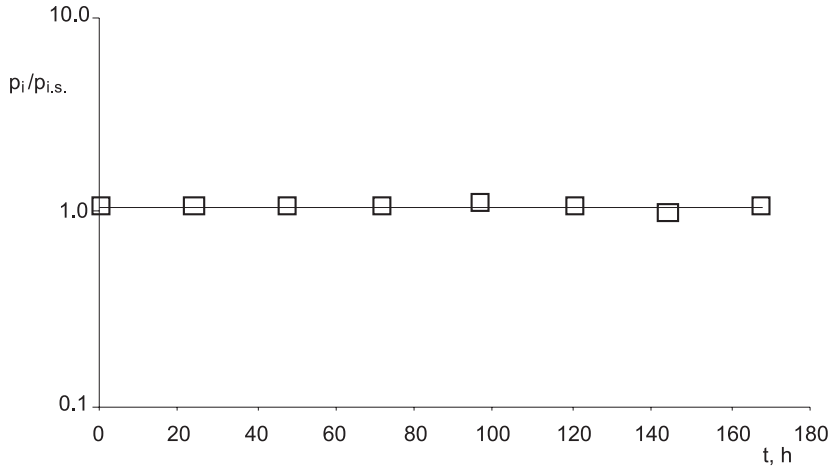

Figure 5. Stability of vitamin B_6 in the hydrochloric acid (0.1 M) determined by the HPLC method

Table 1. Characteristic of the pharmaceutical preparations.

Symbols	Pharmaceutical formulation	Active compounds	Arithmetic mean of tablets weight (g)
FB	tablets	pyridoxine hydrochloride, 5 mg magnesium hydroaspartate, 600 mg	0.6964 ± 0.0039
LB	tablets	pyridoxine hydrochloride, 5 mg magnesium hydroaspartate, 1000 mg	1.3431 ± 0.0023
MB	coated tablets	pyridoxine hydrochloride, 5 mg magnesium lactate dihydrate, 470 mg	0.9290 ± 0.0113
PB	lozenges	pyridoxine hydrochloride, 5 mg magnesium lactatogluconate, 552 mg magnesium hydroaspartate, 297 mg	1.2046 ± 0.0052
SB	enteric-coated tablets	pyridoxine hydrochloride, 5 mg magnesium chloride hexahydrate, 535 mg	0.9368 ± 0.0117

Table 2. Characteristic parameters for the regression equations of the proposed UV methods (zero, first and second derivative of the ratio spectra: A, D^1, D^2) for determination of the pyridoxine hydrochloride.

Parameters	A	D^1	D^2
λ, nm	290	302	308
calibration range, μg/mL	1.0 – 20.0	1.0 – 20.0	1.0 – 20.0
slope (*b*)	0.0427 ± 0.0001	0.592 ± 0.001	0.00604 ± 0.00004
S_b	4.02×10^{-5}	5.98×10^{-4}	1.57×10^{-5}
correlation coefficient (*r*)	0.9999	0.9999	0.9999
LOD, mg/mL	0.050	0.059	0.152
LOQ, mg/mL	0.183	0.197	0.506

Table 3. Determination of pyridoxine hydrochloride in commercial pharmaceutical products using the UV and HPLC methods.

Tablets	Concentration mg	Concentrations found, mg			
		A	D^1	D^2	HPLC
FB	5.00	5.48 ± 0.08	5.32 ± 0.09	5.35 ± 0.07	5.28 ± 0.03
LB	5.00	5.23 ± 0.03	5.10 ± 0.03	5.10 ± 0.03	5.05 ± 0.04
MB	5.00	5.27 ± 0.08	4.78 ± 0.04	4.62 ± 0.04	4.81 ± 0.05
PB	4.00	4.33 ± 0.03	4.08 ± 0.03	3.33 ± 0.02	4.04 ± 0.04
SB	5.00	4.99 ± 0.04	4.89 ± 0.03	4.91 ± 0.03	4.93 ± 0.05

Differential spectra were collected using a UV-VIS spectrophotmeter (Lambda 20, Perkin Elmer) with the range of 220 nm to 350 nm, in a 1-cm layer, with the speed of spectrum registering at 240 nm/min, the spectral width of the radiation leaving the monochromator of 1.00 nm and the increase in wavelength of 1 nm. Hydrochloric acid (0.1 M) was used as a reference sample.

Reversed-phase high performance liquid chromatography (HPLC-RP) was used as a reference method. Chromatograms were registered by means of a liquid chromatograph consisting of: an LC-6A pump (Shimadzu), a UV-Vis SPO-6AV detector (Shimadzu), a C-RGA Chromatopac integrator (Shimadzu) and a Rheodyne 7125 dispenser with a dispensing loop, 100 μL (Berkley). The chromatogra-

Table 4. Application of the standard addition technique to the analysis of vitamin B_6 using the proposed UV methods.

Tablets	Concentration, mg			Recovery %	SD	RSD %
	Added	Method	Found			
FB	0.700	D^1	0.669 ± 0.013	95.6	0.01770	2.64
	0.700	D^2	0.665 ± 0.013	95.0	0.01834	2.76
LB	0.700	D^1	0.700 ± 0.011	100.0	0.01485	2.12
	0.700	D^2	0.716 ± 0.011	102.3	0.01478	2.06
MB	0.700	D^1	0.701 ± 0.009	100.1	0.01228	1.75
	0.700	D^2	0.747 ± 0.009	106.6	0.01243	1.66
PB	0.700	D^1	0.711 ± 0.014	101.6	0.01396	1.96
SB	0.700	A	0.697 ± 0.007	99.5	0.01239	0.70

phy conditions described in the United States Pharmacopoeia USP 24 for a multi-vitamin preparation were used. The stationary phase was LiChrosorb 100 RP-18 column (250 × 4,6 mm, 5 µm; Merck), the mobile phase was a mixture of sodium heptanosulfonate (0.6 g/L) with 10 mL of acetic acid (1.05 kg/L) with a pH 3.0 achieved by means of sodium hydroxide (1 M) – methanol (47:53, v/v). The column was washed with the mobile phase at the speed of 1.5 mL/min. Chromatograms were registered using the analytical wavelength of 280 nm and a methanol solution of methyl 4-hydroxybenzoate (0.5 mg/mL). The retention times of pyridoxine hydrochloride and the internal standard amounted to approximately 2.8 min and approximately 5.3 min, respectively (Fig. 1).

RESULTS

Spectral analysis and standard curves

A solution of vitamin B_6 in hydrochloric acid (0.1 M) with the concentration of 0.15 mg/mL was prepared and the absorbance (A), the first (D^1) and second (D^2) derivative (Figs. 2–4) spectra were collected. The following analytical wavelengths were adopted for the individual types of spectra: A: λ = 290 nm; D^1: λ = 302 nm; D^2: λ = 308 nm. Eight solutions of pyridoxine hydrochloride in hydrochloric acid with concentrations from 1.0 do 20.0 µg/mL were prepared. A, D^1 and D^2 spectra were collected and measurement values were read with the established analytical wavelength by means of the "peak-zero" method. Each measurement value was the mean of three independent measurements. The diagrams of A, D^1 and D^2 absorbances as functions of the vitamin B_6 concentration in hydrochloric acid (0.1 M) are rectilinear and can be described by the following equation: $y = bx$ (Table 2).

Analyte stability

The stability of pyridoxine hydrochloride in hydrochloric acid (0.01 M) was analyzed by means of the HPLC method (Fig. 1). A vitamin B_6 solution (0.525 mg/mL) in hydrochloric acid (0.1 M) was prepared and it was stored at room temperature under uncontrolled conditions. The mixture of the analyzed vitamin B_6 solution and the internal standard solution (1:1, v/v) was applied onto the chromatographic column at appropriate time intervals and the chromatograms were registered. It was assumed that the vitamin B_6 distribution under the analysis conditions was consistent with the first-order reaction described by the following equation:

$$\ln(p_i/p_{i.s.})_t = \ln (p_i/p_{i.s.})_0 - kt,$$

where: k is the reaction rate constant in s^{-1}; t is the time expressed in s.

The equation parameters were calculated with the *b* value being the gradient of the first line. It was found that there is no statistically significant difference between the value of the gradient of the straight line semi-algorithmic dependency ($p_i/p_{i.s.}$) as a time function and zero (Fig. 5). Thus, the solution of pyridoxine hydrochloride solution in hydrochloric acid (0.1 M) is durable at room temperature and it does not undergo decomposition within the analyzed time limits from t = 0 to t = 168 h. The calculated values of $t_{0.1}$ and $t_{0.5}$ amount to 34 and 224 days, respectively.

Determination of vitamin B_6 content using the UV method

The amount of the tested preparations corresponding to 1.5 mg pyridoxine hydrochloride was accurately weighed and 60 mL of hydrochloric acid (0.1 M) was added. The preparation was shaken for 5 min and filled up with the same solvent up to the

volume of 100.00 mL, then mixed and filtered. The UV spectra were collected (A, D^1 and D^2), the measurement value was read using the "peak-zero" method with established analytical wavelengths and the vitamin B_6 content in the average weight of a tablet was calculated (Table 3).

Determination of vitamin B_6 content using the HPLC method

The amount of the tested preparations corresponding to approximately 0.5 mg of pyridoxine hydrochloride was weighed accurately, 9.0 mL of the mobile phase and 1 mL of internal standard solution were added and the mixture was shaken for approximately 5 min and filtered. A hundred mL of each: the prepared extracts and the mixture of the vitamin B6 reference (50 μg/mL) with the internal standard (50 μg/mL) in the mobile phase were applied onto the chromatographic column. Chromatograms were registered and the content of vitamin B_6 was calculated in the average weight of a tablet (Table 3). Nine independent repetitions of the determination were performed.

Evaluation of the accuracy of the UV and HPLC methods

In a mortar, 10 tablets of the tested preparations were crushed, 7.00 mg of pyridoxine hydrochloride was added and the crushing was continued. Next, the vitamin B_6 content was determined using the UV method. The determined amount of excess pyridoxine hydrochloride was calculated as expressed in the average tablet weight as well as the accuracy relative to the actually added excess vitamin (Table 4). Nine independent repetitions of the determination were performed.

DISCUSSION

The aim of the study was to show the possibility of using differential spectrophotometry in the determination of vitamin B_6 in various drug forms and their composition in pharmaceutical preparations. Preparations containing pyridoxine hydrochloride (4–5 mg) as an active substance and various magnesium compounds were selected for the study: hydroaspartate, lactate, lactogluconate, chloride (Table 1). The HPLC method was used as a reference method.

The analysis of the absorbance spectra and its first (D^1) and second (D^2) derivatives made it possible to establish the appropriate analytical wavelengths (A: λ = 290 nm; D^1: λ = 302 nm; D^2: λ = 308 nm). The vitamin B_6 content was determined in the preparations by means of the UV and HPLC methods and the comparison of the results obtained with the values declared by the manufacturer revealed that all preparations (apart from the PB preparation in the determination using the D^2 technique) fall within the limits of ±10% of the declared value (Table 3). Besides, all values obtained in the determination by means of the UV method using absorbance measurements are statistically different from the remaining determinations. The results obtained in this way are heightened compared to the reference selective HPLC method (Table 3). Such differences were observed not only in the results of the vitamin B_6 determination in the SB preparation containing magnesium chloride (Tables 1, 3). In the remaining preparations, magnesium hydroaspartate, magnesium lactate and/or magnesium lactate gluconate were present in addition to pyridoxine hydrochloride. The application of differential spectra made it possible to eliminate this effect and to obtain results which were not statistically different from determinations performed by means of the reference method (HPLC) (Table 3). Therefore, it was decided to determine the accuracy of the UV method using differential spectra based on the determination of excess pyridoxine hydrochloride added to the average tablet weight (0.700 mg). The appropriate absorbance derivative was selected for each preparation: FB, LB and MB – D^1 and D^2; PB – D^1 and for SB – A (Table 4). The results obtained from the evaluation show that the application of the first derivative technique (D^1) effectively eliminates the background effect and that it can be successfully used in the determination of vitamin B_6 in a mixture with organic magnesium compounds.

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